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AIR FORCE PROGRAMS IN FUEL MICROBIOLOGY

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The Air Force first became aware of the existence of microorganisms in hydrocarbon products in 1956 through the efforts of Bakanauskus (ref. 1). During the intervening years, several workers have attempted to isolate the quantitative and qualitative facets of microbial growth and activity in hydrocarbon environments and have achieved somewhat limited success. The efforts of Wulf (ref. 11), Churchill and Leathen (refs. 3 and 4), Klemme and Leonard (ref. 8), Hazzard (ref. 7), DeGray (ref. 5), Dooley (ref. 6), and Powelson (ref. 9) have suggested the magnitude and complexity of the problem which users of turbine engine fuels are encountering. However, the extent of the problem and the causative factors are ill-defined. During a recent inspection tour of the Project BEARS facility at Kindley AFB, Bermuda, the central question was reiterated: What role do microorganisms play in the formation of sludge and the inception of corrosion in JP-4 and Avgas fueling systems? Unfortunately, unequivocal answers to this and other related questions could not be given. In particular, the engineers present pressed for answers to questions such as: What does the microbial count in a sample mean? What organism or organisms are of primary importance?

At the present time, the microbiologist is at a loss to implicate microbial entities in any cause-effect relationship. Other than the obvious appearance of an agglomerate of fungal mycelia, the activities, growth rates under actual field conditions, and effects on environmental composition of biological agents are, at best, very poorly recognized. The most difficult aspect of the problem, and that which the nonbiologist finds most difficult to understand, is the probable functioning of a complex ecological scheme in each hydrocarbon environment. These systems are not static but rather change in time with the kinds and numbers of organisms present. This, in fact, is at the core of the practical problems associated with obtaining experimental data; i.e., a sample obtained in the field represents only one point in a time continuum and hence the organisms isolated from that sample might very well not represent the biotic sequence which could be responsible for any deleterious changes observed in the system. In addition, the isolation

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techniques applied up to this time have certainly been biased in that only a few methods have been utilized which have not favored the appearance of all biochemical types of microorganisms.

Because of the great effect that microbial growth in turbine fuels might have on Air Force logistics and possibly even operational capability, an extensive program was initiated by several Aeronautical Systems Division (ASD) offices to investigate different aspects of the problem. Members of the Biospecialties Section, 6570th Aerospace Medical Research Laboratories, are acting as microbiological consultants to these offices. A certain degree of overlap exists between some of the efforts to be described. This has been purposeful to insure that several potential approaches will be considered.

Eleven contracts have been awarded during calendar year 1962 at a cost of about one million dollars. Pertinent information on these contracts can be found in table 1. Since most of the contracts were initiated only recently, little or no data are available on their progress.

The objective of the work effort initiated by Battelle Memorial Institute is to obtain information for the development of new or modified integral tank coatings and sealants which are resistant to microbial degradation and compatible with the established JP-4 environment. (This effort has presumed, of course, that microorganisms are intimately associated with fuel system problems and must be eliminated from or controlled in this environment.) The Battelle group has divided its investigation into four phases. Phase I concerns a study of biocidal substances which can be added to available sealants and coatings. Only PR-1560 (polyurethane) will be used initially in a screening of approximately 100 additives. The Phase II effort is a study of the materials comprising sealants and coatings which might serve as nutrient sources for selected microorganisms. A wide variety of substances will be examined. The formulation of new sealant and coating systems will be attempted in Phase III. This includes new curing techniques. Finally, Phase IV is concerned with the compatibility of coating and sealant systems with fuel and/or water-soluble biocidal additives. This phase is at present minimal since potassium dichromate is the only substance being considered.

The contract with Monsanto Research is a study of the components of selected fuels and lubricants which either enhance or inhibit microbial growth. In essence, under this program the component fractions of hydrocarbons representing both the most susceptible and least susceptible samples will be individually analyzed for their effects on microbial activity. A total of 25 fuels will be examined and an attempt will be made to correlate the growth (effect) of up to 50 microbial isolates in these fuels with the presence of specific fractions. Additives will also be considered. This effort will hopefully result in information upon which new fuel specifications can be based. In reality, this investigation is directed towards the formulation of so-called "immune" fuels.

TABLE 1

AF CONTRACTS PERTAINING TO MICROBIAL CONTAMINATION OF AIRCRAFT FUELS

CONTRACTOR	CONTRACT NUMBER	TITLE	FUNDS	DATE INITIATED	DURATION	CHIEF INVESTIGATOR	CONTRACT MONITOR	SYMBOL
Battelle Memorial Institute Columbus, Ohio	33(657)-9804	Elastomers for fuel systems containing microorganism controlling additives.	200,000	1 Oct 62	2 years	Mr. C. W. Cooper	Mr. P. House	ASRCNE-1
Monsanto Rsch. Corp., Everett, Massachusetts	33(657)-9814	Effects of microorganisms on the composition of fuels and lubricants.	139,103	13 Aug 62	3 years	Dr. J. O. Smith	Mr. A. V. Churchill	ASRCNL-3
Southwest Rsch. Inst., San Antonio, Texas	33(657)-9762	Development program for investigation, laboratory analysis & evaluation of base fuel handling procedures with regard to microorganism contamination.	66,661	2 Aug 62	1 year	Mr. R. Johnson Dr. F. W. Bieberdorf	Mr. K. F. Stevensen	ASNNSF
Univ. Dayton, Dayton, Ohio	33(657)-9175	Research on advanced aerospace fuels under high mach number flight stresses.	150,000	15 Jun 62	3 years	Dr. Dorothy Nunn	Mr. A. V. Churchill	ASRCNL-3
USA Chem. Corps Biol. Labs., Ft. Detrick, Maryland	MIPR # 33-657-2-RD-264	Study of microbiological contamination.	90,000	13 Jul 62	3 years	Dr. H. M. Hodge	Mr. A. V. Churchill	ASRCNL-3
General Dynamics, Ft. Worth, Texas	33(657)-9181	Mechanical techniques for killing, removing or controlling microorganisms in hydrocarbon fuels.	67,300	1 Jul 62	1 year	Dr. D. J. Pritchard Dr. H. G. Hedrick	Mr. C. R. Martel	ASRMFS-2

TABLE 1 (continued)

USA Eng. Rsch. & Deve. Labs., Ft. Belvoir, Virginia	MIPR # 33(616)61-9 MIPR # 33-657-2-RD-154	Project BEARS.	103,000	29 Dec 61	2 years	Mr. R. Rogowski	Mr. T. Drennen	ASNNSF
General Dynamics, Ft. Worth, Texas	33(657)-8752	Microbiological corrosive effects on structural materials used in aircraft fuel tanks.	150,000	22 Oct 62	18 mos.	Mr. D. C. Wilson Dr. H. G. Hedrick	Mr. B. Ward	ASRCEE-1
Sharpley Labs., Fredericksburg, Va.		Handbook for hydro-carbon microbiology.			1 year	Dr. J. M. Sharpley	Mr. A. V. Churchill	ASRCNL-3
Melpar, Inc., Falls Church, Va.	33(657)-9186	Research on contaminants in aircraft fuel systems and the development of rapid methods for the detection of microbial contaminants.	575,000	15 Dec 62	3 years	Dr. G. Blanchard	Mr. A. V. Churchill	ASRCNL-3
Automation Indust. Torrence, Calif.	33(657)-9731	Applicability of ultra-sonic techniques for evaluation of corrosion in integral fuel tank assemblies of military aircraft.	85,953	17 Aug 62	1 year	Mr. J. B. Ramsey	Mr. E. McKelvey	ASRCEE-1

The third contract, with Southwest Research Institute, is primarily devoted to the operational aspects of the problem, i.e., the effects of contaminated fuel on the fuel handling equipment from bulk storage to the aircraft. The goal of this effort is the establishment of optimal procedures for the control of microbiological contamination (as well as water and other particulate contaminants). This program will combine: (a) analyses of field practices, (b) requisite laboratory studies, and (c) adequate testing procedures resulting in the formulation of new or modified techniques.

The University of Dayton has been awarded a rather large contract which is broad in nature. The research, in a general sense, is concerned with the effects of high Mach number aerospace flight stresses on advanced aerospace fuels. This includes various physicochemical aspects as well as an investigation of microbiological effects. Initially, most of the effort will be given to the study of microbial growth in an aviation fuel environment. As outlined in the technical exhibit, the following program may be implemented:

a. Investigation of the role of microorganisms in the corrosion process under both static and dynamic conditions

b. Investigation of the role of microorganisms in the modification of properties of fuels and lubricants

c. Investigation of environmental requirements for the growth of microorganisms and the conditions required for rapid growth

d. Determination of the level of microorganism contamination required for participation in the corrosion process

e. Evaluation of the activity of water-soluble and fuel-soluble inhibitors on microbiological growth and their effects on fuels and fuel systems

f. Determination of reasons for apparent inherent biostatic properties of some aviation fuels

g. Development of rapid identification techniques for establishing the presence of microorganism contaminants in fuels and lubricants

This is an extensive program and, undoubtedly, all of the above points will not be completely examined. Data obtained from other contractual efforts will be used by the University of Dayton — thus the necessity for writing such an all-encompassing contract.

A most significant study to be conducted at the University of Dayton is an investigation of the ecological relationships in a simulated fuel tank. Not only will this work help to clarify the importance of the sequential appearance of particular microbial entities with respect to environmental effects, but also pertinent information on the inadequacies of current sampling techniques will be obtained. This is probably the first

attempt at a truly controlled study of both qualitative and quantitative factors operating in a JP-4 environment. Our laboratory plans to utilize this set-up to ascertain corrosion rates (with the Magna Corrosometer) and correlate this with the microbiological data.

The next contract to be discussed is a MIPR with Dr. Hodge at the Biological Laboratories, Ft. Detrick. As described in the technical exhibit, this is a "general program for developing information pertaining to microbiological growths and their effects on fuels," including a study of water-soluble and fuel-soluble biocides. Again, this contract is a flexible one, permitting Dr. Hodge and his group quite a bit of latitude in their investigations. Some of the work which has progressed includes microbial identification, effect of dichromate on microbial populations, compatibility of various additives, inhibitory effects of the anti-icing additive (MIL-I-27686), and studies on effects of fuel composition on microbial growth.

Another contract awarded by ASD is that with General Dynamics, Ft. Worth, on "Mechanical Techniques for Killing and/or Removing Microorganisms in Hydrocarbon Fuels." This title is misleading in that it really refers to non-chemical approaches to the problem. The first phase of this contract is a theoretical analysis of all possible "mechanical" techniques which have been used or might be used for the control of microbial growth. These can be considered as either removal techniques or cidal/static techniques. Under the former, one finds methods such as ultrafiltration, electrostatic precipitation, centrifugation, and agglomeration/filtration, while the latter group includes electromagnetic radiations, ultrasonics, electricity, and nuclear radiation. The second phase of this contract is an experimental one in which the recommendations of the theoretical study will be evaluated in the laboratory.

The organisms to be used in this study were selected from a compilation of genera and species reported by various workers to be most frequently associated with microbiological contamination of hydrocarbon fuels. The contractor was advised by ASD to secure the cultures in a JP-4 environment and to keep them in contact with the jet fuel since removal from this environment frequently results in loss of ability to grow in JP-4. The organisms chosen are shown in table 2. General Dynamics/Ft. Worth is currently studying the possibility of using all of these organisms in a composite culture. Their results so far are not too encouraging. As one might suspect, compatibility of such a diverse group might not be feasible.

The literature studies conducted by General Dynamics/Ft. Worth indicate that electromagnetic radiation in the RF (radio frequency) range has a definite lethal effect on microorganisms above that caused by induced temperature rise. Because of the difference in dielectric characteristics between microorganisms and JP-4 (microorganisms are from 75 to 86 percent water, while JP-4 is an excellent nonconductor, its dielectric constant being 2.08 which is stable over a frequency range from 10^4 to 3×10^9 cps), it may be possible to irradiate a stream of fuel with sterilizing levels of RF radiation. The use of nuclear radiation is negated due to the prohibitive

TABLE 2

LIST OF ORGANISMS SELECTED FOR USE IN AF #33(657)-9181
RESEARCH PROGRAM AT GENERAL DYNAMICS, FORT WORTH

Bacteria

Pseudomonas aeruginosa
Pseudomonas fluorescens
Bacillus subtilis
Bacillus cereus
Aerobacter aerogenes
Flavobacterium arborescens
Clostridium sporogenes
Micrococcus radiodurans
Achromobacter cycloclastes
Sphaerotilus natans
Desulfovibrio desulfuricans
Aluminum corrosion organism (two sp.)

Fungi

Cladosporium resinae (or *Hormodendron sp.*)
Aspergillus niger
Spicaria violacea
Penicillium ochrochloron
Alternaria tenuis
Fusarium roseum

Yeast

Rhodotorula rubra

cost of the radiation devices (Cobalt-60 source, reactor, or accelerator) and ancillary equipment. Finally, consideration has been given to ion exchange resins which are compatible with hydrocarbon fuels. These may be useful by removing nutritive ions from both fuel and water phases, and thereby preventing further growth of microbial contaminants.

Another interdepartmental contract is Project BEARS (Bacteriological Effects, Aircraft Refueling Systems). This is a joint Air Force-Army effort conducted by the Engineer Research and Development Laboratories (ERDL), Ft. Belvoir, Virginia. In essence, this program was initiated to investigate the effects of microorganisms growing in JP-4 and Avgas on filter-separator components of the Pritchard refueling system. This effort combines a field study of actual operational equipment with static tests of a variety of fuel-water environments. The physical plant of the Project BEARS facility offers a unique opportunity to verify laboratory data through actual field testing procedures. Data obtained in the laboratory are subject to experimental compromise and frequently are not indicative of field conditions. Only rigidly controlled evaluations in the operational environment can provide valid and meaningful data. This facility duplicates rather than merely simulates operational conditions. The BEARS facility should, therefore, be considered indispensable as the final step in a test procedure prior to incorporation into operational systems. An immediate problem which should be investigated at the Kindley facility is the efficacy of the anti-icing additive as a biocide. In addition, this facility offers the only field evaluation capability for the following critical investigations:

a. Evaluation of biocides and other additives

b. Relationships of microorganisms to corrosion and other degradative processes

c. Compatibility of additives with various components of the ground refueling system

d. Stimulation of microbial growth by materials found in structural components, sealants, and coatings

e. Rapid detection techniques for microbial contamination

A second contract with General Dynamics/Ft. Worth concerns an investigation on the effects of microbiological corrosion on the mechanical properties of materials used in aircraft fuel cell structures. This program is based on the assumption of a direct cause-effect relationship of microbial growth to metallic corrosion. The work effort will consist of three general phases:

a. Preliminary Investigations

1. Methods for corrosion product removal

2. Methods for classifying corrosion pits

3. Examination of ASD-furnished biologically corroded specimens (based on a1 and a2) to be used as standards

4. Selection of microbial corrosion system (based on 7178-T6 aluminum panels)

5. Selection of simulated corrosion system and preparation of test specimens

b. Mechanical Testing (1,488 specimens)

1. Static

2. Dynamic

c. Data Analysis

The program will include the testing of four types of aluminum alloys. The aim of this research is to provide information on microbiologically corroded fuel cell structures pertinent to their serviceability, requirement for repair, or requirement for replacement.

A contract is in the final stages of negotiation with Dr. J. M. Sharpley for the preparation of a definitive handbook on petroleum microbiology. The necessity for such an authoritative reference on this subject is recognized by all who are associated with this facet of microbiology. Dr. Sharpley's book will extend considerably his present manual (ref. 10) as well as the work by Beerstecher (ref. 2) and incorporate the most recent observations of eminent petroleum microbiologists. This effort should result in a standard work which can serve as a guide and source book for future investigations.

The largest of the ASD contracts was awarded to Melpar. One phase of the effort is an extensive investigation of techniques for rapid detection of microorganisms in JP-4. Detection of low levels of contamination prior to the onset of any deleterious effects on the environment is the goal of this study. In addition, techniques must not only be sufficiently reliable but must be practical for routine application. Some of the approaches to be considered are: gas chromatography, electrophoresis, optical rotary dispersion, ultraviolet spectrophotometry, enzyme detection, ESR (electron spin resonance), and coulometric titration. Attempts will be made to devise techniques which are capable of distinguishing corrosion-causing organisms from noncorroding types.

The second phase of the contract is a very broad research study of the relationship of fuel contaminants to: (a) the formation of sludge and slime, (b) the corrosion and degradation of fuel system components, and (c) alterations in chemical and physical properties of jet fuels. Included in this study will be measurements of microbial growth rates in various hydrocarbon environments

and under various conditions as well as investigations of anaerobic-aerobic growth relationships. This program should result in the acquisition of vital basic data pertinent to this problem.

The final contract, although not associated with the microbiological aspects of the problem, is of interest since it is concerned with a unique method of corrosion detection. Automation Industries will conduct a feasibility study on the detection of integral fuel tank corrosion through the application of ultrasonic techniques. The contract requires a detection capability of 0.002 inch in materials ranging from 0.050 inch to 1.50 inch thick. The technique: (a) will not require aircraft defueling, (b) will provide a permanent record, (c) must utilize portable equipments, and (d) will detect the presence, kind, and degree of corrosion. This is, admittedly, a difficult set of requirements. However, the contractor has indicated the current state-of-the-art to be most promising. Successful completion of this contract will permit, eventually, the examination of all integral fuel tank aircraft, resulting in qualitative and quantitative information which can be correlated with chemical, physical, and microbiological data. Thus, it may be possible, as well as operationally acceptable, to maintain a close surveillance of all service aircraft.

Although these contracts cover the significant areas of the total problem, this Laboratory is conducting or will initiate several research efforts to extend or supplement these studies. Since experience has shown that repetition of results is not always obtainable, some experiments will be performed to verify the findings of other workers.

Since several laboratories have found various results with the dichromate ion as a microbial inhibitor, a study on the effects of this substance on microorganism viability and growth was initiated. Several points require clarification. The first effort was concerned with rapid methods of measuring dichromate concentration in an aqueous mineral salts or water bottom environment. Preliminary studies have shown that colorimetric analysis may be applicable. At 500 mμ, the procedure is within 3 percent error, is simple, and similar results have been obtained by different analysts. The utility of centrifugation and glass wool filtration of samples requiring particulate clarification is being studied. This method will enable rapid analysis of field and laboratory samples for correlation with microbiological data.

Another point to be investigated is the difference between sodium and potassium dichromate as microbial inhibitors as well as the difference between chromate and dichromate ions. As discussed with personnel of ERDL, these differences have not been investigated and may account for the discrepancies reported by various laboratories.

Since the biocidal potency of dichromate is questionable, a study of the survival curves of representative species is in order. This will include observations on the emergence of resistant forms, since growth in water bottoms containing dichromate of unknown concentration has been observed.

Dr. Hodge has shown good evidence from field samples that the anti-icing additive has excellent biocidal properties. This has occurred when concentrations in the water bottoms have been greater than 10 percent. In this Laboratory low concentrations of about 1 percent were observed to have no effect. To corroborate Dr. Hodge's findings, the effects of higher levels and also the appearance of resistant forms are being studied. In addition, the compatibility of dichromate and the anti-icing additive will be investigated since reports are at variance on this point.

The examination of fuel and water bottom samples has been confined mainly to aerobic species. With the exception of the anaerobic sulfate reducers, no intensive work has been performed on the presence of anaerobic types. Since the oxygen tension of fuel environments is not high, a detailed study on the existence of anaerobic biocontaminants is certainly warranted. This effort will include the usual anaerobic media as well as aerobic media incubated in an anaerobic environment. Should the data so indicate, corrosion studies will also be implemented.

In an attempt to simulate field conditions, and also as an adjunct to Dr. Nunn's work at the University of Dayton, a study of a section of an integral fuel tank will be conducted in an environmental test chamber. Environmental parameters will be chosen which most closely duplicate actual conditions. Samples will be taken periodically to observe the effects on microbial populations and on the initiation of the corrosion process. The magnitude of this program will be dependent on the availability of test materials and manpower.

In any microbiological program which requires the collection of samples in the field, the time delay between sampling and analysis raises serious questions as to the validity of the resultant observations. Sharpley (ref. 10) has discussed the findings of several workers on the population changes occurring in shipped samples, indicating very extensive differences can result. To a great extent, knowledge of the microbiological contaminants of jet fuels is based upon determinations performed on shipped samples. Some laboratories have studied samples which were as much as two months old. The Biospecialties Section is studying several different media with the intent of designing a field sampling kit. Mr. A. West has evolved a prototype kit which consists of (a) several liquid media dispensed in serum bottles, (b) sterile hypodermic syringes, (c) several agar media in small plastic petri dishes to accommodate Millipore filters, and (d) cotton swabs saturated with Roccal and sealed in cellophane. The final design of this kit together with the Millipore sampling method will enable the acquisition of qualitative and quantitative data which are truly representative of actual conditions.

Dr. Higgins at the University of Dayton has shown that the Bushnell-Haas medium, which has become the standard mineral salts formulation, is far from the most favorable environment for the observation of organisms in JP-4 and associated water bottoms. The criterion used was extent of growth of selected organisms. This study will be pursued in detail with the Dayton group and a mineral salts medium which yields the highest total counts will be selected.

Although all the organisms present in a hydrocarbon environment may not actually grow and thrive in this medium, the variation in chemical parameters of water bottoms (pH can range from 3 or 4 to 9) makes it impossible to select a "standard" aqueous phase. Hence, the medium which promotes the highest level of growth has the best possibility of isolating the majority of contaminants.

Finally, as another index of cause-effect relationships, a study will be initiated on corrosion rates as measured with the Magna Corrosometer and associated probes. This will be conducted in conjunction with the University of Dayton study on population dynamics and should result in some quantitative data.

The combination of the contractual efforts and the in-house research program is a rather intensive and extensive effort. The completion of this program should result in resolution of the problem of microbial contamination in hydrocarbon fuel systems.

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